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PATENT- OG VAREMÆRKESTYRELSEN

METHOD OF PREPARING AN EDIBLE PRODUCT

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FIELD OF THE INVENTION

The present invention relates to a method of preparing an edible product with a low water content by heating a raw material comprising carbohydrate, protein and water.

5 BACKGROUND OF THE INVENTION

E. Tabeke et al. (*J. Agric. Food Chem.*, 2002, 50, 4998-5006) reported that acrylamide is formed during heating of starch-rich foods to high temperatures. The acrylamide formation has been ascribed to the Maillard reaction (D.S. Mottram et al., R.H. Stadler et al., *Nature*, 419, 3 October 2002, 448-449).

10 SUMMARY OF THE INVENTION

According to the invention, the formation of acrylamide during heat treatment in the production of a food product is reduced by treating the raw material with an enzyme before the heat treatment. Accordingly, the invention relates to a method of preparing an edible product, comprising the sequential steps of:

- 15 a) providing a raw material which comprises carbohydrate, protein and water
- b) treating the raw material with an enzyme which is
 - i) an oxidoreductase capable of reacting with a reducing sugar as a substrate and/or
 - ii) an enzyme capable of reacting with asparagine or glutamine (optionally
- 20 substituted) as a substrate, and
- c) heat treating to reach a final water content below 35 % by weight.

DETAILED DESCRIPTION OF THE INVENTION

Raw material and enzyme treatment

The raw material comprises carbohydrate, protein and water, typically in amounts of
25 10-90 % or 20-50 % by weight. The carbohydrate may consist mainly of starch, and it may include reducing sugars such as glucose, e.g. added as glucose syrup, honey or dry dextrose. The protein may include free amino acids such as asparagine and glutamine (optionally substituted).

The raw material may include tubers, potatoes, grains, oats, corn (maize), wheat, nuts,
30 fruits, dried fruit, bananas, sesame, rye and/or rice.

The raw material may be in the form of a dough comprising finely divided ingredients (e.g. flour) with water. The enzyme treatment may be done by mixing (kneading) the enzyme

into the dough and optionally holding to let the enzyme act. The enzyme may be added in the form of an aqueous solution, a powder, a granulate or agglomerated powder. The dough may be formed into desired shapes, e.g. by sheeting, cutting and/or extrusion.

The raw material may also be in the form of intact vegetable pieces, e.g. slices or other pieces of potato, fruit or bananas, whole nuts etc. The enzyme treatment may comprise immersing the vegetable pieces in an aqueous enzyme solution and optionally applying vacuum infusion. The intact pieces may optionally be blanched by immersion in hot water, e.g. at 70-100°C, either before or after the enzyme treatment.

The raw material before heat treatment typically has a water content of 10-90 % by weight and is typically weakly acidic, e.g. having a pH of 5-7.

Oxidoreductase capable of reacting with a reducing sugar as a substrate

The oxidoreductase may be an oxidase or dehydrogenase capable of reacting with a reducing sugar as a substrate such as glucose and maltose.

The oxidase may be a glucose oxidase, a pyranose oxidase, a hexose oxidase, a galactose oxidase (EC 1.1.3.9) or a carbohydrate oxidase which is capable of oxidizing maltose. The glucose oxidase (EC 1.1.3.4) may be derived from *Aspergillus niger* e.g. having the amino acid sequence described in US 5094951. The hexose oxidase (EC 1.1.3.5) may be derived from algal species such as *Iridophycus flaccidum*, *Chondrus crispus* and *Euthora cristata*. The pyranose oxidase may be derived from *Basidiomycete* fungi, *Peniophora gigantean*, *Aphyllorales*, *Phanerochaete chrysosporium*, *Polyporus pinsitus*, *Bierkandera adusta* or *Phlebiopsis gigantean*. The carbohydrate oxidase capable of oxidizing maltose may particularly have a higher activity on maltose than on glucose and may, e.g. be derived from *Microdochium* or *Acremonium*, e.g. from *M. nivale* (US 6165761), *A. strictum*, *A. fusidioides* or *A. potronii*.

The dehydrogenase may be glucose dehydrogenase (EC 1.1.1.47, EC 1.1.99.10), galactose dehydrogenase (EC 1.1.1.48), D-aldohexose dehydrogenase (EC 1.1.1.118, EC 1.1.1.119), cellobiose dehydrogenase (EC 1.1.5.1, e.g. from *Humicola insolens*), fructose dehydrogenase (EC 1.1.99.11, EC 1.1.1.124, EC 1.1.99.11), aldehyde dehydrogenase (EC 1.2.1.3, EC 1.2.1.4, EC 1.2.1.5). Another example is glucose-fructose oxidoreductase (EC 1.1.99.28).

The oxidoreductase is used in an amount which is effective to reduce the amount of acrylamide in the final product. For glucose oxidase, the amount may be in the range 50-20,000 (e.g. 100-10,000 or 1,000-5,000) GODU/kg dry matter in the raw material. One GODU is the amount of enzyme which forms 1 μ mol of hydrogen peroxide per minute at 30°C, pH 5.6 (acetate buffer) with glucose 16.2 g/l (90 mM) as substrate using 20 min. incubation time. For other enzymes, the dosage may be found similarly by analyzing with the appropriate substrate.

Enzyme capable of reacting with asparagine or glutamine (optionally substituted) as a substrate

The enzyme may be capable of reacting with asparagine or glutamine which is optionally glycosylated or substituted with a peptide at the alpha-amino and/or the carboxyl position.

- 5 The enzyme may be an asparaginase, a glutaminase, an L-amino acid oxidase, a glycosylasparaginase, a glycoamidase or a peptidoglutaminase.

The asparaginase (EC 3.5.1.1) may be derived from *Candia utilis* or *Escherichia coli*. The glutaminase (EC 3.5.1.2) may be derived from *Escherichia coli*. The L-amino acid oxidase (EC 1.4.3.2) capable of reacting with asparagine or glutamine (optionally glycosylated) as a
10 substrate may be derived from *Trichoderma harzianum* (WO 94/25574). The glycosylasparaginase (EC 3.5.1.26, aspartylglucosaminidase, N4-(N-acetyl-beta-glucosaminy)-L-asparagine amidase) may be derived from *Flavobacterium meningosepticum*. The glycoamidase (peptide N-glycosidase, EC 3.5.1.52) may be derived from *Flavobacterium meningosepticum*. The peptidoglutaminase may be peptidoglutaminase I or II (EC 3.5.1.43, EC 3.5.1.44).

- 15 The enzyme is used in an amount which is effective to reduce the amount of acrylamide in the final product. The amount may be in the range 0.1-100 mg enzyme protein per kg dry matter, particularly 1-10 mg/kg. Asparaginase may be added in an amount of 10-100 units per kg dry matter where one unit will liberate 1 μ mole of ammonia from L-asparagine per min at pH 8.6 at 37 °C

20 Heat treatment

The process of the invention involves a heat treatment at high temperature to reach a final water content (moisture content) in the product below 35 % by weight, typically 1-20 %, 1-10 % or 2-5 %.. During the heat treatment, the temperature at the surface of the product may reach 110-220°C, e.g. 110-170°C or 120-160°C.

- 25 The heat treatment may involve, frying, particularly deep frying in tri- and/or di-glycerides (animal or vegetable oil or fat, optionally hydrogenated), e.g. at temperatures of 150-180°C. The heat treatment may also involve baking in hot air, e.g. at 160-310°C or 200-250°C for 2-10 minutes, or hot-plate heating.

Edible product

- 30 The process of the invention may be used to produce an edible product with low water content from raw materials containing carbohydrate and protein by heat treatment, typically starchy food products fried or baked at high temperatures. Examples of such edible products are potato products, potato chips (crisps), French fries, hash browns, roast potatoes, breakfast cereals, crisp bread, muesli, biscuits, crackers, snack products, tortilla chips, roasted nuts, rice
35 crackers (Japanese "senbei"), wafers, waffles, hot cakes and pancakes.

EXAMPLES

Example 1: Production of potato snacks

Recipe:

Tap water	40 g
Potato flakes dehydrated	52.2 g
Potato starch	5.8 g
Salt	2 g
Enzyme (glucose oxidase, pyranose oxidase or <i>M. nivale</i> carbohydrate oxidase)	120 GODU

Dough Procedure:

- 5 The potato flakes and potato starch are mixed for 30 sec in a mixer at speed 5. Salt and enzyme are dissolved in the water. The solution is adjusted to 30°C +/- 1°C. Stop mixer, add all of the salt/enzyme solution to flour. The dough is further mixed for 15 min.
 Place mixed dough in plastic bag, close bag and allow the dough to rest for 15 min at room temperature.
- 10 The dough is then initially compressed for 60 sec in a dough press.
 The dough is sheeted and folded in a noodle roller machine until an approx. 5-10 mm dough is obtained. The dough is then rolled around a rolling pin and the dough is allowed to rest for 30 min in a plastic bag at room temperature. The dough is sheeted further to a final sheet thickness of approx 1.2 mm.
- 15 Cut the sheet into squares of approx 3 x 5 cm.
 Sheets are placed in a frying basket, placed in the oil bath and fried for 60 sec at 180°C. Hold the noodle basket at a 45° angle and let the product drain until oil stops dripping. Remove the products from the basket and leave them to cool on dry absorbent paper.

CLAIMS

1. A method of preparing an edible product, comprising the sequential steps of:
 - a) providing a raw material which comprises carbohydrate, protein and water
 - b) treating the raw material with an enzyme which is
 - i) an oxidoreductase capable of reacting with a reducing sugar as a substrate or
 - ii) an enzyme capable of reacting on asparagine or glutamine (optionally substituted) as a substrate, and
 - c) heat treating to reach a final water content below 35 % by weight, with the exception of a process where the raw material includes potato, and the enzyme treatment is done with glucose oxidase as the only enzyme.

2. A method of preparing an edible product, comprising the sequential steps of:
 - a) providing a raw material which comprises carbohydrate, protein and water
 - b) treating the raw material with an enzyme which is
 - i) a pyranose oxidase, a hexose oxidase, a galactose oxidase, a carbohydrate oxidase which is capable of oxidizing maltose, a dehydrogenase capable of reacting with a reducing sugar as a substrate or
 - ii) an enzyme capable of reacting on asparagine or glutamine (optionally substituted) as a substrate, and
 - c) heat treating to reach a final water content below 35 % by weight

3. A method of preparing an edible product, comprising the sequential steps of:
 - a) providing a raw material for the edible product which comprises grains, oats, corn (maize), wheat, nuts, fruits, dried fruit, bananas, sesame, rye and/or rice,
 - b) treating the raw material with an enzyme which is
 - i) an oxidoreductase capable of reacting with a reducing sugar as a substrate or
 - ii) an enzyme capable of reacting on asparagine or glutamine (optionally substituted) as a substrate, and
 - c) heat treating to reach a final water content below 35 % by weight

4. A method of preparing an edible product, comprising the sequential steps of:
 - a) providing a raw material which comprises carbohydrate, protein and water
 - b) treating the raw material with an enzyme combination comprising
 - i) an oxidoreductase capable of reacting with a reducing sugar as a substrate and
 - ii) an enzyme capable of reacting on asparagine or glutamine (optionally substituted) as a substrate, and

c) heat treating to reach a final water content below 35 % by weight,

5. The method of any preceding claim wherein the oxidoreductase capable of reacting with a reducing sugar as a substrate is a glucose oxidase, a pyranose oxidase, a hexose oxidase, a galactose oxidase (EC 1.1.3.9) or a carbohydrate oxidase which is capable of oxidizing maltose.
6. The method of any preceding claim wherein the enzyme capable of reacting on asparagine or glutamine (optionally substituted) as a substrate is an asparaginase, a glutaminase, an L-amino acid oxidase, a glycosylasparaginase, a glycoamidase (peptide N-glycosidase) or a peptidoglutaminase.
- 10 7. The method of any preceding claim wherein the raw material is in the form of a dough and the enzyme treatment comprises mixing the enzyme into the dough and optionally holding.
8. The method of any of claims 1-6 wherein the raw material comprises intact vegetable pieces and the enzyme treatment comprises immersing the potato pieces in an aqueous solution of the enzyme.
- 15 9. The method of any preceding claim wherein the raw material comprises a potato product.